2357-Pos
Epitaxial Assembly Kinetics of Mutant Amyloid Beta 25-35 Fibrils
Miklos S. Kellermayer[1], Andrea Horvath[2], Unige Murvai[1], Katalin Soos[3], Botond Penke[3].
[1]Semmelweis University, Budapest, Hungary, [2]University of Pecs, Pecs, Hungary, [3]University of Szeged, Szeged, Hungary.
Amyloid ß25-35 (Aß25-35) is a toxic fragment of Alzheimer's beta peptide. We have previously shown that Aß25-35 forms a trigonally oriented network on mica by epitaxial growth mechanisms. To establish chemical reactivity, we use a mutant peptide, Aß25-34_N27C, in which Cys27 is accessible in the mica-associated fibril. In the present work we explored the kinetics of epitaxial assembly of the mutant fibrils at different peptide and KCl concentrations by using in situ time-resolved AFM. We measured the number and length of Aß25-25_N27C fibrils per unit scan area as a function of time. At low (<4 mM) KCl concentration both the number and the growth rate of the fibrils increased with increasing peptide concentration. Increasing KCl concentration decreased the number of fibrils bound to the mica surface, and above 32 mM KCl fibril formation was completely abolished even at high peptide concentrations. The epitaxial assembly of Aß25-35_N27C fibrils is thus a diffusion-limited process inhibited competitively by KCl. The process proceeds via two steps: binding of the peptide to the free fibril end and to the mica surface. While peptide concentration affects both processes, K+ ions compete with peptide binding to the mica surface. By modulating peptide and KCl concentration the complexity of the Aß25-35_N27C network can be finely tuned.

2358-Pos
Micro Mechanical Properties of Model Alzheimer's Beta Amyloid Fibrils
Benjamin J. Soukup[1], Patricia Soto[2].
[1]Montana State University, Bozeman, MT, USA, [2]Creighton University, Omaha, NE, USA.
Protein nanofibrils are the ordered end product in the protein self-association process. Firstly recognized as linked to amyloid diseases, including Alzheimer's, they are currently identified as remarkably stable states accessible to almost any polypeptide chain. Interestingly enough, protein nanofibrils made of the same polypeptide building blocks adopt different ordered three dimensional structures. The purpose of this study is to investigate via all-atom molecular dynamics simulations the micromechanical properties of model Alzheimer's B(9-40)-amyloid fibrils that display different quaternary structure. These structures consisted of a three-fold symmetric model[1] and a striated-ribbon pattern[2]. We found that the Young's modulus value of the model fibril structures both fall in the range of 10^8 Pa, consistent with values found in the literature, with the striated-ribbon structure being more flexible than the three-fold symmetric model. We propose possible relationships between the geometry of the fibril and the interactions that govern structure stability.
Footnotes
[1] Paravastu AK, Leapman RD, Yau WM, Tycko R. Molecular structural basis for polymorphism in Alzheimer's beta-amyloid fibrils. Proc Natl Acad Sci U S A. 2008 Nov 25;105(47):18349-54.
[2] Petkova AT, Yau WM, Tycko R. Experimental constraints on quaternary structure in Alzheimer's beta-amyloid fibrils. Biochemistry. 2006 Jan 17;45(2):498-512.

2359-Pos
Surface Effects on Mutant Aβ Aggregation
Elizabeth A. Yates, Justin Legleiter.
West Virginia University, Morgantown, WV, USA.
Alzheimer's Disease (AD) is a late onset neurodegenerative disease, that is part of a group of diseases commonly classified as 'protein misfolding' diseases. Such diseases are defined by the rearrangement of specific proteins to non-native conformations, promoting the formation and deposition of toxic, nanoscale aggregates within tissues or cellular compartments. A pathological hallmark of AD is the development of neuritic amyloid plaques comprised predominantly of aggregated β-amyloid (Aβ). Aggregated Aβ has several forms, including spherical oligomers, annular aggregates, protofibrils, and amyloid fibrils. The precise mechanisms by which protein aggregates are toxic remains unclear. In kinetic and thermodynamic studies, conformational changes can be induced in proteins encountering surfaces and may play a role in nucleating and inducing aggregate formation. A series of point mutations, clustered around the 22 amino acid of Aβ, alter the aggregation kinetics of Aβ and are associated with familial forms of AD. These mutations alter the charge properties of Aβ, providing an excellent system to determine the role electrostatic surface interactions play in Aβ aggregation. We hypothesize that the altered charge of mutant Aβ peptides will influence their interactions with model surfaces, resulting in altered aggregation both kinetically and morphologically. To test this hypothesis, we used *in situ* atomic force microscopy to directly observe the aggregation of wild type (WT) and mutant Aβ peptides on mica under near physiological conditions. These results were compared with aggregates formed under free solution conditions. While the mutations altered Aβ aggregation kinetics under free solution conditions, the respective aggregate morphologies were indistinguishable. However, aggregation of Aβ WT and mutants on the mica surface differed not only kinetically but also morphologically. These studies provide insight into the valuable role surface chemistries may play in Aβ aggregation and the onset of familial AD.

2360-Pos
Templated Lateral Fibril Growth, an Alternative Mechanism for Beta-Amyloid Fibril Formation
Muriel Arimon[1], Fausto Sanz[1], Ernest Giralt[2,3], Natàlia Carulla[4,2].
[1]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain, [2]Institute for Research in Biomedicine (IRB), Barcelona, Spain, [3]Organic Chemistry Department, University of Barcelona, Barcelona, Spain, [4]ICREA Researcher at Institute for Research in Biomedicine (IRB), Barcelona, Spain.
One of the major pathological hallmarks of Alzheimer's disease (AD) is the deposition of senile plaques in specific areas of the brain. Amyloid fibrils formed by the amyloid-β peptide (Aβ) are the major components of the senile plaques. Aβ amyloid fibril formation is a complex process that starts with the aggregation of monomeric protein molecules into transient oligomeric species, which grow and reorganize into the characteristic amyloid fibrils. Among the different forms of Aβ, the 42-residue fragment ($A\beta_{42}$) is the most strongly linked to the ethiology of AD. Although one of the main unresolved issues in the field of Aβ aggregation and its relation to AD concerns the molecular mechanisms leading to cytotoxicity, it is crucial to describe the main players and the pathways involved in this process.
In this work we present a single molecule resolution fibril growth assay based on two variants of $A\beta_{42}$ that can be differentially detected. The presence of a biotin moiety on one of them enables its detection by means of a primary and secondary antibody (the latter conjugated to a gold nanoparticle), and the subsequent visualization by transmission electron microscopy. By mixing non-labeled fibrillar seeds with labeled monomeric $A\beta_{42}$ we have been able to monitor the process of amyloid fibril formation and fibril growth. The observation of the precise location (with nanometric resolution) of the newly incorporated $A\beta_{42}$ monomers, has revealed an alternative mechanism for $A\beta_{42}$ fibril growth based on the lateral association of $A\beta_{42}$ monomers to pre-existing fibrils, using those as templates for the new born protofilament.

2361-Pos
Structural Transitions Associated with the Assembly Dynamics of Transthyretin Amyloid Protofibrils
Ricardo H. Pires[1], Árpád Karsai[2], Maria J. Saraiva[3], Ana M. Damas[3], Miklós S. Z. Kellermayer[1].
[1]Semmelweis University, Budapest, Hungary, [2]University of Pécs, Pécs, Hungary, [3]IBMC & ICBAS, University of Porto, Porto, Portugal.
Transthyretin (TTR) is a serum protein involved in the transport of thyroxine and co-transport of retinol. Several neurodegenerative diseases are associated with the aggregation of TTR into amyloid fibrils, but the molecular mechanisms of the assembly remain unknown. Here we used AFM to identify intermediates along the assembly-disassembly pathway of TTR amyloid protofibrils. By using dynamic force spectroscopy, we also characterized the mechanical response of protofibrils at different growth stages before and after their partial disassembly.
During TTR assembly, annular oligomers with 15.8 ± 2.3 nm in diameter appeared after 3-5 days of incubation. These annular oligomers displayed a tendency to associate laterally, forming linear structures that preceded the appearance of amyloid protofibrils. In other proteins, similar annular oligomeric structures have been implicated in amyloid cytotoxicity, but it remains unclear to which extent they represent an "on pathway" intermediate. Upon solvent exchange, dramatic morphological rearrangements occurred leading to the partial disassembly of protofibrils. During disassembly, annular oligomers with 7.0 ± 0.6 nm appeared, suggesting that the annular arrangement is a common motif in the TTR assembly and disassembly pathways. Force spectroscopy of both native TTR and early protofibrils contained a sawtooth pattern with ~4 nm periodicity. Conceivably, this pattern corresponds to successive structural transitions related to the sequential unfolding of the ß-strands within the TTR monomer. Force spectra of TTR protofibrils also revealed a time-dependent increase in the length of the manipulated structure, indicating that the axial association between monomers stabilizes with time. Thus, stabilization of intermonomeric contacts appears to be a much slower process than that of mere assembly, possibly involving additional structural rearrangements of the monomer within the protofibrils.